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Biochimica et Biophysica Acta 1762 (2006) 269–275

BIOCHIMICA ET BIOPHYSICA ACTA

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Involvement of Kupffer cells in lipopolysaccharide-induced rapid accumulation of platelets in the liver and the ensuing anaphylaxis-like shock in mice

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Received 8 February 2005; received in revised form 28 November 2005; accepted 29 November 2005
Available online 5 January 2006

Abstract

Intravenous injection of *Klebsiella* O3 lipopolysaccharide (LPS) into BALB/c mice induces an anaphylaxis-like shock within minutes. Using 5-hydroxytryptamine as a marker for platelets, we previously suggested that a rapid platelet accumulation in the liver and lung precedes the shock, and that a complement-dependent platelet-degradation is involved in the shock. Here, we examined (i) the effect of platelet-depletion (using an anti-platelet monoclonal antibody) on the shock and (ii) the contribution of macrophages to the platelet-accumulation in those organs. LPS-induced platelet-accumulations in the liver and lung were confirmed by immunostaining. In platelet-depleted mice, the shock was largely prevented. The number of F4/80-positive macrophages was much greater in liver than in lung, and the hepatic macrophages were largely lost in mice given clodronate-encapsulated liposomes. In mice treated with such liposomes, both the LPS-induced accumulation of platelets in the liver (but not in the lung) and the shock were largely prevented, and repopulation of hepatic macrophages restored these LPS-induced responses. These results suggest that (i) platelets are indeed involved in the shock, (ii) Kupffer cells mediate the hepatic platelet accumulation, and (iii) preventing this hepatic accumulation can largely prevent rapid shock being induced by LPS (at the dose used here).

Keywords: Platelet; Lipopolysaccharide; Liver; Lung; Shock; Macrophage

1. Introduction

Traditionally, platelets have been considered to play major roles in hemostasis. However, recent studies suggest that another fundamental physiological role of platelets lies in the defense against invasion by foreign organisms, and that platelets are involved in inflammatory reactions during immune responses [1–4].

Lipopolysaccharide (LPS) is a constituent of the cell wall of gram-negative bacteria with the potential to induce various non-specific immune responses [5,6]. Using 5-hydroxytryptamine (5HT) as a marker for platelets, we previously observed a unique response by platelets to LPS [7–11]. Briefly, within a few minutes of the intravenous injection into mice of a certain type of LPS, platelets accumulate in the liver (at lower doses of LPS) and in both the liver and lung (at higher doses). Thus, the liver is more sensitive to LPS than the lung in terms of the accumulation of platelets. In addition to its dependence of the dose of LPS, this platelet response also depends on the structure

Abbreviations: Clo-Lip, clodronate-encapsulated liposomes; HPA, hepatic platelet accumulation; 5HT, 5-hydroxytryptamine; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; PPA, pulmonary platelet accumulation

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doi:10.1016/j.bbadis.2005.11.010

of the LPS, the strain of the mouse, and activation of the lectin pathway of the complement system. At their maximal levels, the LPS-induced pulmonary platelet accumulation (PPA) and hepatic platelet accumulation (HPA) reach about 80% and 30%, respectively, of the total numbers of circulating platelets. When the response is weak or the LPS dose is low, the platelets that have accumulated in these organs soon return to the circulation. When the response is strong or the LPS dose is high, degradation (or destruction) of platelets occurs in the lung [7,9] and possibly in the liver [8]. However, the mechanism by which platelets accumulate in these organs remains to be clarified.

It is known that platelets produce and/or store a variety of inflammatory substances, such as thromboxane A_2, 5HT, adrenaline, histamine, and PAF [3,4], and these substances are known to induce shock at pharmacological doses. Indeed, within 5 to 10 min of an intravenous LPS injection, anaphylaxis-like rapid shock occurs when there is a degradation of platelets in the lung and/or liver [7–9]. An inhibitor of complement C5 prevents both the degradation of platelets and the development of the shock, although it does not reduce the PPA and HPA themselves [8,11]. In addition, in mice deficient in complement C5, platelets accumulate in the liver and lung in response to LPS, but soon return to the circulation without degradation, and there is no rapid shock [8,11]. These results suggest that it is the *degradation* of platelets in the lung and/or liver that is the cause of the anaphylaxis-like shock (i.e., the PPA and HPA themselves are not the direct cause of the rapid shock). However, it is not clear which organ, the lung or the liver, makes the greater contribution to the induction of the shock.

In addition to the rapid platelet responses described above, LPS induces another type of HPA, which occurs some hours after its injection [7,9,12]. We tentatively call this "slow HPA" as opposed to the "rapid HPA" described above. The slow HPA is induced even by the intraperitoneal injection of a very low dose of LPS. Interestingly, the LPS-induced slow HPA has been shown to be largely absent in mice depleted of their Kupffer cells (hepatic phagocytic macrophages) [13] in experiment making use of the finding that phagocytic macrophages can be selectively depleted by an intravenous injection of liposomes encapsulated with clodronate [14].

In the present study, as an approach to clarifying the mechanism by which platelets accumulate rapidly in the liver and lung, we examined the effects of depletion of phagocytic macrophages on the *Klebsiella* O3 LPS-induced rapid HPA, rapid PPA, and anaphylaxis-like shock in BALB/c mice.

2. Materials and methods

2.1. Animals and materials

Male BALB/c mice obtained from the animal facility of our university were used in the experiments. Clodronate (dichloromethylene bisphosphonate) was purchased from Sigma (St. Louis MO, USA). A rat monoclonal antibody to mouse F4/80 antigen was purchased from Serotec (Kidlington, UK). The hybridoma cells employed to produce a monoclonal rat anti-mouse platelet antibody, Pm-1, were provided by Dr. T. Nagasawa (Division of Hematology, University of Tsukuba, Japan) [15]. This antibody was produced in the peritoneal cavity in BALB/c nude mice inoculated with the hybridoma cells, and a preparation (IgG fraction) of the antibody was obtained by precipitation with ammonium sulfate and dialysis of the precipitant. Control mouse IgG was prepared by precipitation with ammonium sulfate. LPS from the *Klebsiella* O3 strain LEN-1 (S type) was prepared using the phenol–water method [16], dissolved in sterile saline, and injected intravenously via the tail (0.1 ml per 10 g body weight). The injection of LPS was carried out at a room temperature of 26–28 °C. All experiments complied with the *Guidelines for Care and Use of Laboratory Animals in Tohoku University*. Experimental protocols and doses of these reagents are described in the text or in the legend to the figure or table relating to each experiment.

2.2. Depletion and detection of macrophages

Clodronate-encapsulated liposomes (Clo-Lip) have been shown to deplete phagocytic macrophages, but not dendritic cells and neutrophils [14]. A suspension of Clo-Lip was prepared by a method similar to that used by Van Rooijen and his coworkers [14], as described previously [17]. Briefly, 75 mg of phosphatidylcholine and 11 mg of cholesterol were dissolved in chloroform (20 ml) in a round-bottomed flask (1000 ml). The thin film that formed on the walls of the flask after rotary evaporation at 37 °C was dispersed by gentle shaking for 10 min in 10 ml of clodronate solution (200 mg/ml) in 10 mM sodium phosphate buffer (PBS, pH 7.4). This suspension was kept for 2 h at room temperature, then sonicated for 3 min (50 Hz) and kept for another 2 h. The resulting liposomes floating on the aqueous phase were collected by Pasteur pipette, then suspended in 10 ml of PBS and centrifuged at 5000×*g* for 30 min. The precipitated liposomes were finally suspended in 4 ml of PBS; this preparation is hereafter referred to as the "original clodronate-liposomes". This original suspension was diluted (as described in the text) with PBS and the resulting suspension was injected intravenously via the tail into mice at 0.2 ml/mouse.

2.3. Platelet count

Two or three drops of blood from each decapitated mouse were directly collected into a pre-weighed test tube containing 1.0 ml of 4 mM EDTA in 0.01 M PBS (pH 7.0). The tube plus blood was weighed, and the volume of blood was estimated from the weight of the blood. The number of platelets was then ascertained using a cell counter, Sysmex SF-3000 (Toa Medical Electronics Co. Ltd., Kobe, Japan).

2.4. Estimation of HPA and PPA

HPA and PPA were estimated by measuring 5HT as described previously [7–11]. Briefly, after collection of the blood for measuring the platelet count (see above), the next two or three drops of blood from the same mouse were collected into another pre-weighed tube containing 3 ml of 0.4 M $HClO_4$ containing 0.2% *N*-acetylcysteine–HCl and 4 mM EDTA-2Na. After reweighing, the platelets were destroyed by sonication, and each tube was cooled in an ice bath. The lungs and liver were rapidly removed after collection of the blood, and the 5HT in these tissues was also extracted with 0.4 M $HClO_4$ containing *N*-acetylcysteine–HCl and EDTA-2Na. The 5HT extracted from the blood and tissues was separated by column chromatography, and measured fluorometrically as previously described [12].

2.5. Immunostaining of macrophages

Macrophages in livers and lungs were detected by immunohistochemical staining of F4/80 antigen using its monoclonal antibody (see above). F4/80 is expressed specifically on the surface of these cells [18,19]. Specimens of lungs and livers were fixed in 4% paraformaldehyde phosphate buffer and embedded in paraffin. Serial sections from tissue blocks were deparaffinized and immersed in ethanol containing 0.3% hydrogen peroxide. After treatment with 3% skim milk for 30 min, the sections were incubated with rat anti-mouse F4/80 antigen monoclonal antibody (20 μg/ml) at 4 °C overnight. The sections were allowed to react with peroxidase-conjugated ant-rat IgG polyclonal antibody (Histofine Simple Stain MAX-PO) (Nichirei, Tokyo Japan) for 30 min, and the reaction products were visualized by immersing the sections for 2 to 3 min in 0.03% diaminobenzidine solution containing 2 mM hydrogen peroxide. Nuclei were lightly stained with methyl green.

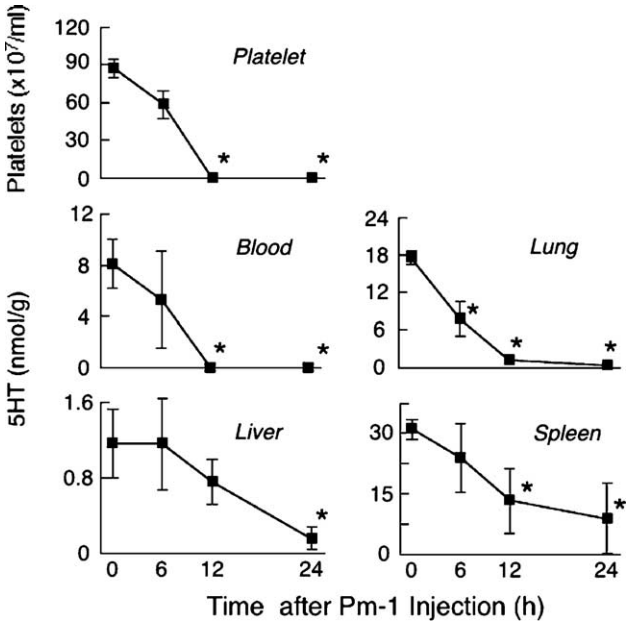

Fig. 1. Effects of Pm-1 on platelet count and on 5HT in the blood and tissues. Pm-1 (10 mg/kg) was injected subcutaneously and the blood and tissues of the mouse were removed at the time-points indicated. Each value is the mean±S.D. for 4 mice. *$P<0.01$ vs. time 0.

2.6. Immunostaining of platelets

Platelets accumulated in livers and lungs were detected using the anti-platelet antibody Pm-1 (see above). Sections of lungs and livers were embedded in Tissue-Tek OCT Compound (Sakura Finetechnical, Tokyo, Japan), then quick-frozen in a mixture of acetone and dry ice. Serial cryostat sections cut at 5 μm thick from each frozen block were fixed in 4% paraformaldehyde phosphate buffer for 10 min, then washed in cold phosphate-buffered saline. After treatment with normal rabbit and goat sera for 20 min, the sections were incubated with Pm-1 (6.3 μg/ml) at 4 °C overnight. The sections were allowed to react with peroxidase-conjugated anti-rat IgG polyclonal antibody (see above) for 30 min, and the reaction products were visualized by immersing the sections for 2 to 3 min in 0.03% diaminobenzidine solution containing 2 mM hydrogen peroxide. Nuclei were lightly stained with hematoxylin.

Table 1
Effect of platelet depletion by Pm-1 on LPS-induced anaphylaxis-like shock

Treatment	Shock score
cIgG→LPS	3, 3, 3, 4, 4
Pm-1→LPS	0, 0, 0, 0, 1

Two groups of mice ($n=5$ each) were subcutaneously injected with control IgG (cIgG) or Pm-1 (10 mg/kg each). Then, 12 h later, LPS was injected intravenously (1 mg/kg). The shock score indicates the maximum points allocated for the signs of shock observed within 30 min of the LPS injection.

2.7. Scoring of shock

The maximal score given to the severity of the rapid anaphylactoid shock in each mouse within 30 min of LPS injection was recorded as described previously [11]. The scoring of the shock was as follows: 0 (no symptoms of shock), 1 (staggering), 2 (crawling and prostration), 3 (prostration and weak convulsions), 4 (prostration and strong convulsions), and 5 (death).

2.8. Statistical analysis

Experimental values are given as the mean±standard deviation (SD). The statistical significance of differences was analyzed using a Bonferroni post hoc test, P values less than 0.05 being considered to indicate significance.

3. Results

3.1. Effects of platelet depletion on the LPS-induced rapid shock

A single subcutaneous injection of Pm-1 at 10 mg/kg markedly reduced both the platelet count and 5HT concentration in blood after 12 h (Fig. 1). In addition, the 5HT in the liver and lung had disappeared almost completely at 24 h after the Pm-1 injection, while the magnitude of the depletion in the spleen was smaller. These results indicate that 5HT in the blood, liver, and lung is contained mostly in platelets. As the half-life of free 5HT in the blood of mice is less than 10 s [20], the measured amount of 5HT in the liver and lung may represent almost entirely the amount of 5HT contained in the platelets located within these organs. Despite this profound thrombocytopenia, there was no

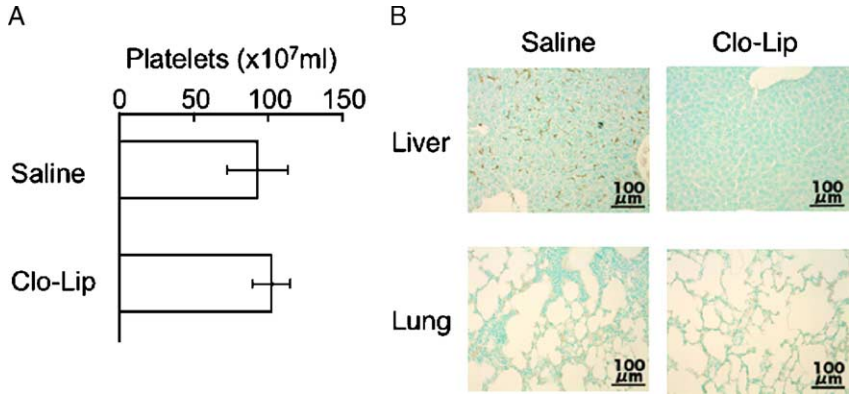

Fig. 2. Effects of Clo-Lip on platelets and macrophages. (A) Saline or Clo-Lip (diluted 8-fold) (0.2 ml/mouse) was injected intravenously, and 2 days later the blood of the mouse was taken and subjected to platelet counting. Each value is the mean±S.D. for 4 mice. (B) The livers and lungs of the mice were subjected to immunostaining with F4/80 antibody, and representative results are shown. There are many F4/80-positive cells (brown) in the liver of saline-treated mice, but they are almost absent from the liver of Clo-Lip-treated mice. In the lung, however, almost no F4/80-positive cells were detected in either saline-treated or Clo-Lip-treated mice. Noted that the magnification for microscopy is the same for liver and lung.

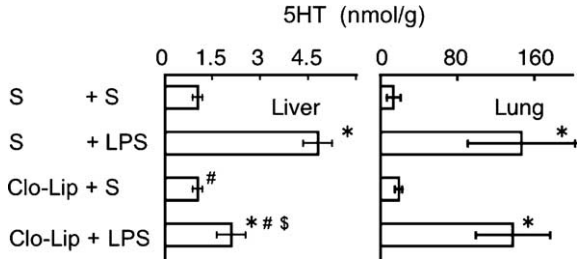

Fig. 3. Effects of Clo-Lip on LPS-induced rapid HPA and PPA. Saline (S) or 8-fold-diluted Clo-Lip was injected intravenously (0.2 ml/mouse). Two days later, LPS was injected intravenously (0.5 mg/kg). The mice were killed 5 min after the LPS injection. Each value is the mean±S.D. for 4 mice. *$P<0.01$ vs. S+S, $^{\#}P<0.01$ vs. S+LPS, $^{\$}P<0.05$ vs. Clo-Lip+S.

apparent illness in the mice, and platelet counts recovered progressively from 2 days after the injection of Pm-1 (data not shown). The severity of the shock induced by LPS (1 mg/kg) was greatly reduced in mice given Pm-1 at 12 h before the injection of LPS (Table 1). These results indicate that 5HT can be a marker of platelet translocation from the blood to the liver and lung, and that platelets are indeed involved in the LPS-induced anaphylaxis-like shock.

3.2. Effects of Clo-Lip on platelets and hepatic macrophages

Our previous data indicated that at higher concentrations, Clo-Lip reduces the number of platelets as well as that of phagocytic macrophages [21]. Thus, in the present study we injected an 8-fold diluted suspension of the original Clo-Lip. This injection did not reduce the number of platelets found in the blood 2 days later (Fig. 2A), but it largely eliminated F4/80-positive cells (macrophages) from the liver (Figs. 2B and 5). However, in the lung, F4/80-positive cells were scarce even in control mice (Fig. 2B) and the number detected was insufficient to allow us to evaluate the effect of Clo-Lip (it should be noted that in Fig. 2, the magnification for microscopy is the same for liver and lung). In the present study, we used saline-injected mice as a control, because liposomes that contain PBS alone modulate the responses of macrophages [22].

3.3. Effects of Clo-Lip on the rapid HPA, PPA and anaphylaxis-like shock

As shown in Fig. 3, an intravenous injection of LPS (0.5 mg/kg) induced a marked increase in 5HT in the liver and lung at 5 min. Immunostaining of platelets with Pm-1 antibody demonstrated that this 5HT elevation in the liver and lung reflects HPA and PPA (Fig. 4). The rapid increase in 5HT in the liver was largely absent in mice given Clo-Lip at 2 days before the LPS injection (Fig. 3). However, no significant effect of Clo-Lip was seen in the lung (Fig. 3). It should also be noted that the increase in 5HT in the lung in response to LPS was about 10-fold greater than that in the liver in terms of nmol/g, although F4/80-positive cells in the lung were so few that we could hardly detect them (Fig. 2B).

Another notable point is that although much less 5HT accumulated in the liver than in the lung in terms of nmol/g, the total amount of 5HT accumulated in the liver was not smaller than that in the lung. From the 5HT values shown in Fig. 3 and the weights of the liver (about 1.40 g) and lung (about 0.10 g), the total amounts of 5HT in the liver and lung were calculated as 4.5 nmol and 3.8 nmol, respectively.

LPS at 1 mg/kg induced significant shock signs including staggering, crawling, prostration, and convulsions (Table 2). These symptoms developed 6–8 min after the injection of LPS (i.e., the peak platelet accumulations in the liver and lung preceded these signs of shock). The severity of the shock was completely abrogated in mice given Clo-Lip (Table 2).

3.4. Recovery of the response to LPS in Clo-Lip-treated mice

Mice treated with Clo-Lip displayed a progressive recovery in their number of F4/80-positive cells (Fig. 5). In the liver, these cells showed a full restoration to their initial level at 20

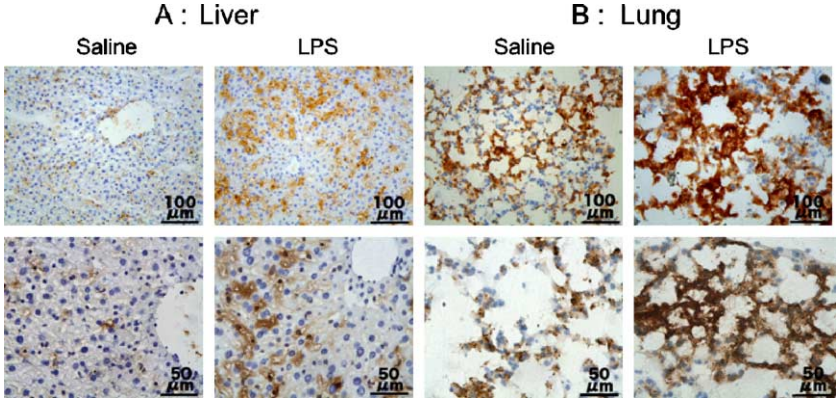

Fig. 4. Histochemical demonstration of platelets in the liver (A) and lung (B) at 5 min after an intravenous injection of saline or LPS (0.5 mg/kg). Livers and lungs were subjected to immunostaining with Pm-1 antibody, and representative results are shown at two different magnifications. The strength of Pm-1 staining (brown) was much greater in LPS-injected mice. In the liver, the staining is localized around sinusoidal vessels. In the lung, it is localized to capillaries in both saline- and LPS-treated mice. Note that there is significant Pm-1 staining in the lung even of saline-treated mice, in line with the observation that the normal level of 5HT is much higher in the lung than in the liver (compare the 5HT levels in the lung and liver at time 0 in Fig. 1). It was also notable that capillaries in the lung of LPS-treated mice were much dilated.

Table 2
Effects of macrophage depletion by Clo-Lip on LPS-induced anaphylaxis-like shock

Treatment	Shock score
Saline→LPS	3, 3, 3, 4, 4
Clo-Lip→LPS	0, 0, 0, 0, 0

Two groups of mice ($n=5$ each) were intravenously injected with saline or Clo-Lip (diluted 8-fold, 0.2 ml/mouse). Then, 2 days later, LPS was injected intravenously (1 mg/kg). The shock score indicates the maximum points allocated for the signs of shock observed within 30 min of the LPS injection.

days after the treatment, and powerful shock responses to LPS were observed at 20 days, but not at 12 days, after the Clo-Lip treatment (Table 3). Moreover, the HPA response observed at 12 days was small, but that at 20 days was as strong as in non-Clo-Lip-treated mice (Fig. 6).

4. Discussion

The findings in the present study may be summarized as follows. (i) In mice whose platelet count had been reduced by Pm-1, the LPS-induced rapid shock was prevented almost entirely. (ii) The existence of LPS-induced HPA and PPA was confirmed by immunostaining. (iii) In control mice, F4/80-positive cells were abundant in the liver but hardly detectable in the lung, and these hepatic F4/80-positive cells were largely lost after injection of Clo-Lip. (iv) In mice treated with Clo-Lip, both HPA (but not PPA) and the anaphylaxis-like rapid shock induced by LPS were largely prevented. (v) The level of F4/80-positive cells in the liver was restored at 20 days (but not at 12 days) after Clo-Lip treatment, and the initial magnitude of the HPA response to LPS was also restored at 20 days (but not at 12 days). These results are discussed in the following paragraphs.

As described in Introduction, LPS induces a biphasic accumulation of platelets in the liver (i.e., a rapid HPA followed by a slow HPA) [7,9]. Previously, we demonstrated that F4/80-positive cells in the liver (hepatic macrophages; i.e., Kupffer cells) are required for the slow HPA [13]. In the present study, F4/80-positive cells in the liver were shown to be involved in the rapid HPA, too, indicating that Kupffer cells are a prerequisite for both the rapid and the slow HPA. However, the molecular mechanisms underlying these two HPA responses to LPS may be different, even if Kupffer cells are important for both responses.

Table 3
Recovery of shock responses to LPS after treatment with Clo-Lip

Treatment	Shock scores	
	Days after Clo-Lip treatment	
	12 days	20 days
Saline→LPS	3, 3, 4, 4, 4	3, 3, 4, 4, 5
Clo-Lip→LPS	0, 1, 1, 1, 2	3, 4, 4, 4, 4

Four groups of mice ($n=5$ each) were intravenously injected with saline or Clo-Lip (diluted 8-fold, 0.2 ml/mouse). Then, 12 or 20 days later, LPS was injected intravenously (1 mg/kg). The shock score indicates the maximum points allocated for the signs of shock observed within 30 min of the LPS injection.

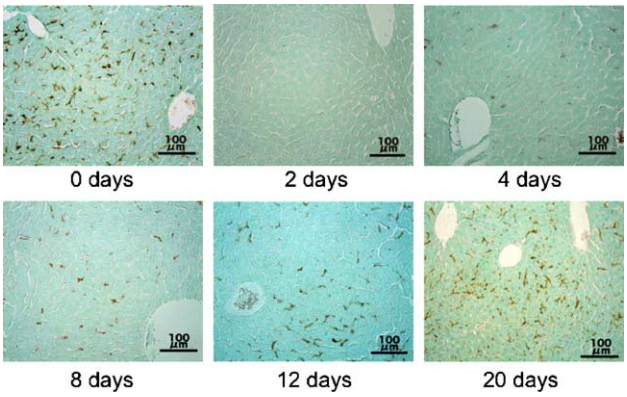

Fig. 5. Recovery in F4/80-positive cells in the liver after Clo-Lip-treatment. Clo-Lip (diluted 8-fold) (0.2 ml/mouse) was injected intravenously, and livers were taken at the indicated times after the Clo-Lip treatment. The livers were subjected to immunostaining with F4/80 antibody, and representative results are shown. The livers had nearly recovered their initial level of F4/80-positive cells (brown) at 20 days after the Clo-Lip injection.

In the present study, the involvement of platelets in the LPS-induced anaphylaxis-like rapid shock was confirmed by a platelet-depletion experiment. However, as described in Introduction, we think that although the HPA and PPA themselves are not the direct cause of the shock, the *degradation* of platelets in the liver and/or lung is involved in the rapid shock. The magnitude of the HPA is smaller than that of the PPA in terms of nmol 5HT per g of tissue. However, as calculated in Results, the *total* amount of 5HT (i.e., the total number of platelets) accumulated is quite similar between liver and lung, or perhaps slightly greater in liver than in lung. In addition, a low dose of *Klebsiella* O3 LPS (20 μg/kg) induces a maximal accumulation of 5HT in the liver without its accumulation in the lung [8]. At larger doses of this LPS (more than 20 μg/kg), the 5HT accumulation increases dose-dependently in the lung (up to 500 μg/kg), but decreases dose-dependently in the liver [8]. In the present study, the prevention of HPA alone (by Kupffer cell depletion) completely abolished the rapid shock. These results indicate that both the accumulation of platelets and their ensuing degradation are more sensitive to LPS in the liver than in the lung, and that a prevention of HPA is sufficient to abolish the LPS-induced rapid shock (at least at the dose of LPS used in the

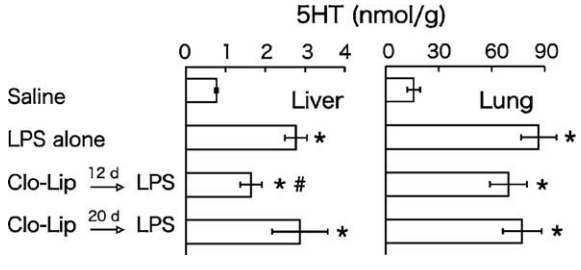

Fig. 6. HPA and PPA responses to LPS at 12 and 20 days after Clo-Lip-treatment. Mice were separated into 4 groups at day 0 (4 mice in each group). Clo-Lip (8-fold-diluted) was given intravenously (0.2 ml/mouse) to two groups (to one group at day 0 and to another group at day 8). At day 20, saline or LPS (0.5 mg/kg) was injected intravenously, and mice were killed by decapitation at 5 min after the injection. Each value is the mean±S.D. for 4 mice. *$P<0.01$ vs. S, $^{\#}P<0.05$ vs. LPS alone, $^{\$}P<0.05$ vs. Clo-Lip (12 days)→LPS.

present study). However, it is likely that larger doses of LPS would induce rapid shock by inducing platelet degradation in the lung even when HPA is prevented by depletion of Kupffer cells, although we have not done this experiment.

F4/80 and BM8 are markers for mature macrophages [23]. Yamamoto et al. [24] reported that in BALB/c mice (as used in our study), BM8-positive cells returned to their normal level by day 10–14 after Clo-Lip-treatment. A similar, but somewhat later, recovery was noted in our study: a full recovery of F4/80-positive cells was seen at 20 days (but not at 12 days) after Clo-Lip injection. This might be due to the use of different markers: in other words, the functional restoration of Kupffer cells may take longer than that of the BM8 maker.

After LPS injection, 5HT accumulated in a much greater amount in the lung than in the liver in terms of nmol/g (Fig. 3), suggesting that platelets accumulate in much greater numbers in the lung than in the liver in terms of g tissue weight. However, the number of F4/80-positive macrophages was very small in the lung, and there was no detectable effect of Clo-Lip on the LPS-induced PPA, suggesting that macrophages are not involved in the mechanism underlying PPA. At present, we have little data to explain the mechanism underlying PPA. However, it was notable that platelets were already present at a significant level in the lung of unstimulated mice (compare the 5HT levels in the lung and liver at time 0 in Fig. 1 and note the strength of Pm-1 immunostaining in the lung of saline-treated mice in Fig. 4). Hence, we speculate that the platelets already present in the lung may somehow be involved in the further accumulation of platelets in this organ. Indeed, platelets mutually stimulate each other to form an aggregation (i.e., pre-existing platelets in the lung and platelets in the blood may mutually stimulate each other, resulting in their extensive accumulation within the lung). Incidentally, pretreatment of mice with a muramyldipeptide (MDP, the minimal structural moiety of bacterial peptidoglycan for various immunological activities) augments both the platelet response and the anaphylaxis-like shock induced by LPS [9]. In addition, such shock can be induced in C3H/HeJ mice possessing a mutation in the Tlr4 gene [25,26], suggesting that the platelet response to LPS is not mediated by Toll-like receptor 4, a representative pathogen-recognition molecule [6].

Instead, we earlier proposed the hypothesis that the lectin pathway that forms C3 convertase from C4 and C2 is involved in the rapid HPA and PPA induced by LPS, and that C5 may be involved in the degradation or destruction of platelets [8]. However, we have no data to suggest how C3 convertase might induce HPA and PPA. Recently, Hoffmeister et al. [27] reported that the interaction between CR3 (complement iC3b receptors) on Kupffer cells and clustered GPIb on chilling-activated platelets results in the hepatic clearance of platelets when chilled platelets are intravenously injected into mice. It would be of interest to examine whether such a mechanism is involved in the "rapid HPA". However, at present, we have insufficient information to guide us towards the molecular mechanism underlying the HPA, or the PPA, induced by LPS.

Finally, approximately 200,000 patients develop gram-negative sepsis each year in the USA [28]. These patients may develop acute respiratory distress syndrome (ARDS, about one-quarter of the patients) or multiple organ failure (MOF) including hepatitis. It has been suggested that in addition to neutrophils and macrophages, platelets may be involved in the pathology of ARDS [29] and MOF [30]. The present findings on HPA and PPA may provide information concerning the possible roles played by platelets in these diseases.

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